MARKED UP VERSION OF AMENDMENTS

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

1. (Thrice Amended) A [DNA] complex comprising semi-purified or pure SV40 VP1 capsid protein or a mixture of VP1 and at least one other SV40 capsid protein; and a constituent selected from the group consisting of:

a) an exogenous substantially histone-free DNA, or an exogenous substantially histone-free DNA encoding an exogenous protein or peptide product, or an exogenous substantially histone-free DNA encoding RNA;

b) a vector comprising any of the exogenous substantially histone-free DNAs of a);

c) an exogenous RNA, or an exogenous RNA encoding an exogenous protein or peptide product;

d) a vector comprising any of the exogenous RNAs of c); or

e) [an exogenous protein or peptide product; or

f)] antisense RNA, ribozyme RNA or any RNA or substantially histone-free DNA which inhibits or prevents the expression of undesired protein or proteins in said mammalian cell;

and further comprising operatively linked elements sufficient for one or more of the following:

(i) replication of said constituent;

(ii) expression of said constituent; and

(iii) prevention of expression of said undesired protein or proteins;

in said mammalian cell.

6. (Thrice Amended) A complex according to Claim 1 wherein said constituent is:

(a) exogenous circular or linear substantially histone-free DNA;

(b) exogenous circular or linear substantially histone-free DNA encoding a protein or peptide product; or

(c[d]) exogenous circular or linear substantially histone-free DNA encoding RNA.

7. (Thrice Amended) A complex according to Claim 6 wherein said substantially histone-free DNA is DNA which encodes a protein or peptide product, wherein said protein or peptide product is not made or contained in said cell prior to infection with the construct, or is substantially histone-free DNA which encodes a protein or peptide product, wherein said protein or peptide product is made or contained in said cell in an amount insufficient for proper cell function prior to infection with the construct, or is substantially histone-free DNA which encodes a protein or peptide product, wherein said protein or peptide product is made or contained in said cell in a form inadequate for proper cell function prior to infection with the construct, or encodes a RNA.

18. (Thrice Amended) A method for the *in vitro* construction of SV40 viruses or pseudoviruses comprising exogenous substantially histone-free nucleic acid comprising the following steps:

a) allowing a semi-purified or pure SV40 VP1 capsid protein or a mixture of VP1 and at least one other SV40 capsid protein to self-assemble into SV40-like particles; and

b) bringing the SV40-like particles assembled in step (a) into contact with said exogenous substantially histone-free nucleic acid to give *in vitro* constructed viruses, or into contact with a vector comprising said exogenous substantially histone-free nucleic acid to give pseudoviruses.

20. (Twice Amended) A method according to Claim 18 wherein in step (a) at least one other SV40 protein, preferably SV40 agnoprotein, is added to the mixture of said SV40 capsid protein or proteins and said exogenous substantially histone-free nucleic acid.

35. (Thrice Amended) A method for the *in vitro* construction of SV40 pseudoviruses comprising exogenous antisense RNA, or ribozyme RNA or RNA or substantially histone-free DNA which inhibits or prevents the expression of undesired protein or proteins in a mammalian cell, comprising the following steps:

a) allowing a semi-purified or pure SV40 VP1 capsid protein or a mixture of VP1 and at least one other SV40 protein to self assemble into SV40-like particles and

b) bringing said SV40-like particles obtained, in step (a) into contact with said exogenous antisense RNA, or ribozyme RNA, or RNA or substantially histone-free DNA which inhibits or prevents the expression of undesired proteins in a mammalian cell, to give *in vitro* constructed SV40 pseudoviruses.

37. (Twice Amended) A method according to Claim 35 wherein in step (a) at least one other SV40 protein, preferably SV40 agnoprotein, is added to the mixture of SV40 capsid protein or proteins and the exogenous antisense RNA or ribozyme RNA or RNA or substantially histone-free DNA.

43. (Twice Amended) A method of transforming [providing] a substantially histone free DNA, RNA, antisense RNA, ribozyme RNA, protein or peptide product into a cell comprising infecting said cell with [to a patient in need of such product by administering to said patient an effective amount of] the construct of Claim 1.

45. (Twice Amended) A composition comprising [as an active ingredient] an effective amount of the complex [construct] of Claim 1 in a pharmaceutically-acceptable carrier.

46. (Twice Amended) A composition comprising [as an active ingredient] an effective amount of the infected cells according to Claim 41, in a pharmaceutically-acceptable carrier.

-- 47. (New) A complex comprising semi-purified or pure SV40 VP1 capsid protein or a mixture of VP1 and at least one other SV40 capsid protein, and an exogenous protein or peptide product. --

REMARKS

Claims 1-2, 4-13, 16-20, 22-37, 41-43 and 45-46 are currently pending in the application. Claim 44 is cancelled, and Applicants reserve the right to pursue this claim in a continuing or other application. Claims 1, 6-7, 18, 20, 35, 37, 43 and 45-46 are amended. The amendments find support in the specification and claims as filed, and such support is discussed in the relevant sections below. No new matter is added by these amendments.

Applicants' representatives, Doreen M. Hogle and Joyce C. Hersh, thank the Examiner for the telephonic discussion of April 18, 2001, in which the outstanding rejections were discussed. The claim amendments proposed during that discussion have been made, and are explained below.

Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 1-2, 4-8, 10-13, 16-20, 22-26, 28-37 and 41-46 stand rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter not described in the specification in such a way as to enable one of ordinary skill in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

The Examiner reiterates that the specification "makes clear the necessity of having an *ori* sequence in each nucleic acid which is encapsidated in the claims SV40 protein capsid structures." The Examiner pointed to statements made on pages 19 and 20 of the specification.

Applicants provided a Declaration under 37 U.S.C. § 1.132, by Orly Ben-Nun Shaul, executed February 4, 2001, providing data showing that the *ori* element, while convenient for use in an assay system, was not actually required for packaging.

An Advisory Action was mailed from the U.S. Patent and Trademark Office on May 15, 2001. In that Action, the Examiner responded to the Declaration, stating that "[t]here is insufficient evidence to evaluate the claim that an ori is not required to practice the claimed invention. There is no explanation of how the contradictory statements of the specification are resolved."

Applicants respectfully submit that the Examiner did not afford proper weight to the Declaration of Ms. Orly Ben-Nun Shaul. Although opinions not based on any scientific principles are of little evidentiary value, expert declarations grounded in logic and scientific reasoning are entitled to fair weight. *E.g.*, compare *Ex parte George*, 21 U.S.P.Q.2d 1058, 1062 (Pat. & Trademark Off. Bd. Pat. Inf. 1991) (an unsupported opinion, although entitled to

consideration, is of limited evidentiary value), with *Ex parte Taaksley*, 37 U.S.P.Q.2d 1382, 1385 (Pat. & Trademark Off. Bd. Pat. Inf. 1995).

To rebut an expert declaration that provides statements of fact or is based on logic and scientific reasoning, the Examiner must articulate adequate reasons. See, *Tanksley*, at 1385 (the Examiner erred in summarily dismissing the declaration which was based on a logical analysis and scientific reasoning and supported by references); see also *In re Alton*, 76 F.3d 1168, 1175 (Fed. Cir. 1996) (the Examiner erred in dismissing the declaration and providing only conclusory statements, which did not rebut the declaration because the declaration contained statements of fact addressing the issue). Even an opinion of a non-expert, but if skilled in the art, is entitled to fair weight so long as the opinion is "reasonably pertinent to the particular problem." *Ex parte Dussaud*, 7 U.S.P.Q.2d 1818, 1819 (Pat. & Trademark Off. Bd. Pat. Inf. 1988).

In the instant case, Ms. Shaul holds a B.Sc. and a M.Sc., and works in the laboratory of inventor Dr. Ariella Oppenheim. The Declaration by Ms. Shaul is based on sound scientific reasoning and is explained by factual examples and supported by laboratory results. For instance, to support the statement that *ori* is not required for *in vitro* packaging, she provides methods of a laboratory procedure, in which packaging was performed with DNA of the pHaMDR1 plasmid, which does not contain the SV40 *ori*. The plasmid was packaged at the same efficiency as plasmids containing the *ori* sequence. This is a factual statement, with experimental evidence provided, not a mere conclusory statement of opinion.

Furthermore, the "contradictory statements of the specification" are not contradictory if the specification is considered as a whole. Page 5, lines 13-16, for instance, states that "[t]he constructs of the invention **may** comprise SV40-derived *ori* DNA sequence" (emphasis added). Page 13, lines 6-7 state that "[t]he constructs of the invention **may optionally** comprise SV40-derived *ori* DNA" (emphasis added). Page 17, lines 12-15 states that "[i]n the method of the invention, SV40-derived *ori* DNA sequence **may be** added" (emphasis added).

The passages cited by the Examiner (at pages 19 and 20 of the specification) do not contradict these statements that *ori* is optional. Page 19, lines 15-23, for instance, describes in the present tense, a single potential embodiment, not the entire invention. Likewise, the statement on page 20, lines 19-22 simply points out an additional embodiment, in which *ses* is removed, leaving SV40 sequences of 100 bp in size, even if the *ori* is left in. Applicants had already stated (on pages 5, 13 and 17) that *ori* is optional. Subsequent text simply builds on this premise, and shows what other embodiments are also contemplated. The Examiner has taken the

descriptions of embodiments from pages 19 and 20 out of context, ignoring clear statements (on pages 5, 13 and 17) that *ori* is optional.

Applicants therefore respectfully submit that the specification be considered in its entirety, and that the declaration evidence supplied by Ms. Shaul be reconsidered with the weight legally due the factual statements presented therein. When the proper weight is given to the evidence, the rejection cannot be maintained.

Claims 43-44 also stood rejected under 35 U.S.C. § 112, first paragraph, as containing subject matter not described in the specification in such a way as to enable one of ordinary skill in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

While not agreeing with the Examiner, solely to speed prosecution, Applicants are willing to cancel or amend claims 43-44. In the telephonic conversation of April 18, 2001, Applicants agreed to cancel claims 43-44. In the present Reply, however, Applicants have cancelled Claim 44, and offer an amended Claim 43 that they sincerely hope will obviate the rejection. Should the Examiner disgree or if substantive examination is required to review the amendments, Applicants representatives are willing to discuss further amendments or cancellations.

Applicants respectfully request that in view of the amendment of Claim 43 and the cancellation of Claim 44, the rejection on this basis be reconsidered and withdrawn.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claims 1-2, 4-13, 16-17 and 34 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention.

The Examiner states that Claim 1, from which the other claims depend, contains an internal inconsistency in that section "e)" provides a constitutent that is an "exogenous protein or peptide product", and that the "DNA construct" of the preamble may be left without an element of DNA.

While not agreeing with the Examiner, solely to speed prosecution, Applicants have amended Claim 1 as discussed with the Examiner, to delete "DNA" from the preamble.

In the Examiner's Advisory Action, which was mailed from the U.S. Patent and Trademark Office on May 15, 2001, the Examiner stated that Claim 1 encompassed a protein

composition without DNA, and that this constituted new matter, and raised new issues which required additional searching and new grounds for rejection. Applicants have further amended Claim 1 to remove the phrase that was in subpart "e)", "an exogenous protein or peptide product". Former section "f)" is now section "e)".

Applicants have also included herewith a new Claim 47, which contains the subject matter of Section "e)" of Claim 1. This new claim is support throughout the specification, including at page 4, lines 11-24; page 11, line 13 to page 12, line 2; page 15, lines 8-9.

Applicants submit that these amendments address the Examiner's concerns, and that the claims are allowable. Applicants respectfully request that the rejection on this basis be reconsidered and withdrawn.

Claims 45-46 were also rejected, the Examiner believing that the term "active ingredient" reads more broadly than is understood by those in the pharmaceutical arts, and that the term is vague and indefinite.

Although not specifically discussed in the telephonic conversation of April 18, 2001, Applicants have amended claims 45-46 to delete this phrase.

Claim Rejections Under 35 U.S.C. § 102(b)

Claims 1-2, 4-7, 9-10-12, 16-20, 22-25, 27-34 and 41-42 are rejected under 35 U.S.C. § 102(b), in view of Christensen *et al.* (of record) and also in view of Colomar *et al.* (of record). Applicants argued in the Reply to the previous Office Action that the DNA of Christensen *et al.* was "nucleoprotein", not naked DNA. The Examiner noted that "naked DNA" was not claimed, and that the anticipatory rejection could be avoided by addition of such a limitation.

A key element in the present invention is the packaging of substantially histone-free DNA, *i.e.*, DNA without histones. In contrast, the particles produced by Colomar *et al.* contained histones. The absence of histones renders the constructs of the invention novel over Colomar *et al.*

Colomar *et al.* used (page 2784, first paragraph) "purified supercoiled polyoma virus DNA" and incubated this DNA with "100-fold molar excess of completely dissociated SV40 as for the analysis shown in Fig. 4", that is in 1M salt solution. This is the standard procedure for dissociating histones from DNA, as the mixture contained histones that were originally present in the SV40 before dissociation. As explained by Colomar *et al.* (page 2782, second column,

second paragraph), the ionic strength was increased to 1M salt in order "to dissociate the DNA and protein subunits of disrupted SV40." These protein subunits are the histones which remained associated with the DNA as nuclear protein (NP) complexes, in the particles disrupted at 0.15 M NaCl (see Fig. 1). As expected, at 1M salt, the NP complexes were fully dissociated. As shown by the authors (Fig. 4a), the physical properties of the DNA were identical to those of free, naked DNA. Therefore the "completely dissociated SV40" mixture contained SV40 capsid proteins, SV40 DNA and free histones.

Colomar *et al.* then added free polyoma DNA, anticipating that part of the polyomavirus DNA will "become associated with histones and SV40 capsid proteins" (page 2784, first column, end of first paragraph, emphasis added). In order to reconstitute the particles, they then lowered the salt concentration by stepwise addition of buffer without any salt. As shown by the authors, this procedure is "based on a method previously shown to reconstitute nucleosome core particles from histones and DNA" (page 2779, last two lines, emphasis added). Thus, their reconstituted particles contained DNA complexed with histone proteins. The process is described in detail, for the reconstituted SV40 particles, in the Discussion section (page 2785, third paragraph). The same process was used for encapsidation of polyomavirus DNA. Thus, although purified supercoiled DNA was added to the disrupted SV40, NP complexes were formed with the histones present in the incubation mixture. It is therefore clear that the re-assembled virus particles did contain histones and were not histone-free.

Applicants have amended Claim 1 to recite that the DNA is "substantially histone free". Support for this amendment can be found at page 33, line 12 of the specification. Applicants respectfully submit that the claims, as amended, are free of the cited art, and respectfully request that the rejection on this basis be reconsidered and withdrawn.

Claims Rejections Under 35 U.S.C. § 103(a)

Claims 1-2, 4-13, 16-20, 22-37 and 41-46 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Christensen *et al.* or Colomar *et al.*, each in view of Carswell *et al.* (of record), Oppenheim *et al.* (*J. Virol.*, 1992, of record), U.S. Pat. No. 5,863,541, Szczylik *et al.* (of record).

The particles that Colomar *et al.* reconstituted with polyomavirus DNA were not infective. The authors state (page 2784, first column, last 3 lines) that "[the infectivity] was found to be low, about the same as that of naked polyomavirus DNA (unpublished results).

SV40 can bind to and enter mouse cells, so the reason for this low infectivity is unclear." From this statement it is clear that no infective polyomavirus particles were produced. Therefore, Colomar *et al.* does not teach now the produce particles with heterologous DNA that facilitates gene delivery into cells.

Even if Colomar *et al.* were successful, their products would have been inappropriate for transfer of DNA into cells, and therefore useless. In contrast, see "transfer" of DNA at page 2, line 1 and page 3, line 25 through page 4, line 5 of the specification, and "infected cells" at page 8, line 17. The putative infective particles with heterologous DNA would be produced in admixture with reconstituted SV40. As both particles are of the same size, density and surface proteins, they would be non-separable from each other. Thus the hypothetical infectious particles produced by the method of Colomar *et al.* would always be contaminated by SV40. The problem of contamination of SV40 particles is solved by the invention described herein. No SV40 DNA is present in the complexes, as the invention is based on the utilization of recombinant SV40 proteins.

Applicants therefore respectfully submit that the claims as amended are patentable in view of either Christensen *et al.* or Colomar *et al.*, and therefore patentable in view of Carswell *et al.*, Oppenheim *et al.*, U.S. Pat. No. 5,863,541, or Szczylik *et al.* Applicants therefore respectfully request that the rejection on this basis be reconsidered and withdrawn.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (781) 861-6240.

Respectfully submitted,

HAMILTON, BROOK, SMITH & REYNOLDS, P.C.

By _____

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Lexington, Massachusetts 02421-4799
Dated: July 12, 2001

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	Ziv Sandalon, Ariella Oppenheim and Amos Oppenheim		
Application No.:	09/068,293	Group:	1636
Filed:	May 6, 1998	Examiner:	W. Sandals
For:	IN VITRO CONSTRUCTION OF SV40 VIRUSES AND PSEUDOVIRUSES		

CERTIFICATE OF MAILING

I hereby certify that this correspondence is being deposited with the United States Postal Service with sufficient postage as First Class Mail in an envelope addressed to Assistant Commissioner for Patents, Washington, D.C. 20231

on 7-12-01 (Date) Melissa Bertolino (Signature)

xxxxxx Melissa Bertolino

Typed or printed name of person signing certificate

DECLARATION OF ORLY BEN-NUN-SHAUL, M.Sc., UNDER RULE 132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Orly Ben-Nun-Shaul, of 16 Hamelri Boulevard, Jerusalem, Israel, declare and state that:

1. I received a B.Sc. in Biology from the Hebrew University of Jerusalem in 1986, and a M.Sc. in Microbiology from the Hebrew University in Jerusalem in 1988. Since 1988, I have been working in the Department of Hematology at the Hadassah University Hospital.

2. I have read the above-referenced application "In Vitro Construction of SV40 Viruses and Pseudoviruses" by inventors Ziv Sandalon, Ariella Oppenheim and Amos Oppenheim, U.S.S.N. 09/068,293, filed May 6, 1998.

3. I am presently working in Dr. Ariella Oppenheim's laboratory, and have conducted research with Dr. Oppenheim regarding the subject matter of U.S.S.N. 09/068,293. Discussed below are experiments and results demonstrating that the *ori* sequence is not required for *in vitro* packaging in the constructs described in U.S.S.N. 09/068,293.

4. The packaging experiment was performed with DNA of the plasmid pHaMDR1, which does not contain the SV40 *ori* element, nor any other SV40 sequence (Kane, S.E. *et al.*, 1988, *Mol. Cell. Biol.* 8:3316-3321), as illustrated in the attached restriction map. As shown below, the experiment clearly demonstrated that this plasmid was packaged at exactly the same efficiency as a series of plasmids that do contain SV40 *ori*, as illustrated in Table 2 of the patent application (USSN 09/068,293).

5. Nuclear extracts of Sf9 cells were prepared according to Schreiber (Schreiber, E., *et al.*, 1989, *Nucl Acids Res.* 15:6419-6436), from cells infected with recombinant baculovirus expressing SV40 VP1. One microliter of nuclear extracts (protein concentration 4.8μg/μl) was mixed by vortexing with 1μg DNA in a total volume of 4μl and placed at 37°C for six hours. $CaCl_2$ and $MgCl_2$ were added to final concentrations of 100μM and 8mM respectively, in a total volume of 6μl, and the reactions were incubated for one additional hour on ice. DNase I digestion was performed using 0.5 unit of enzyme for 10 minutes on ice, and stopped by the addition of EDTA to a final concentration of 5mM.

6. The DNase I treatment was used to remove DNA which was not stably packaged. The reaction products were assayed for infectious units (IU) on CV1-PD monolayers, grown in Dulbecco's modified Eagle's medium with 10% FBS, using a standard SV40 infection protocol (CV1-PD is a sub-line of CV1, the parental line of CMT4). Sub-confluent monolayers were incubated with the packaging mixture for 120 minutes at 37°C, with occasional agitation, followed by the addition of fresh medium. The number of infective centers obtained for a 6μl reaction mixture was used in computing the titer of IU/ml.

7. The procedure yielded infectious units with pHaMDR1. The experiments showed that packaging pHaMDR1 with nuclear extracted containing VP1 yielded 96 infectious

centers per 6µl reaction, equivalent to 1.6×10^4 infectious units (IU) per ml. Thus, the *ori* sequence, while convenient for use in the assay system as it facilitates DNA amplification in the infected cells, is not necessary for packaging.

8. I further declare that all statements made in this Declaration of my own knowledge are true and that all statements made on information and belief are believed to be true. Moreover, these statements were made with the knowledge that willful false statements and the like made by me are punishable by fine or imprisonment, or both under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Orly Ben-Nun-Shaul

Orly Ben-Nun-Shaul

4.2.01

Date